Comm.
Dr. Bing
Dr. Cattell
Dr. Sommers

PHARMACOLOGY

#629D
#629C-6/1/72-5/31/73
#629BR1-6/1/71-5/31/72
#629B-6/1/70-5/31/71
#629A-11/1/68-10/31/69
#629-11/1/67-10/31/68

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022

Application for Renewal of Research Grant

~~Application for Research Grant~~

JAN 29 1973

Date: January 22, 1973

1. Name of Investigator(s): (include Title and Degrees)

Carlton K. Erickson, Associate Professor of Pharmacology and Toxicology, B.S., M.S., Ph.D.

2. Institution & Address:

School of Pharmacy
University of Kansas
Lawrence, Kansas 66044

3. Short Title of Project:

Effects of Nicotine on Free Acetylcholine in the Hippocampus During Learning

4. Proposed Starting Date: June 1, 1973 (Anniversary of original grant)

5. Anticipated Duration of this Specific Study: One Year

6. Brief Descripton of Objectives or Specific Aims:

There are no changes in the objectives or specific aims of the project. However, the applicant is confident of receiving a Special Fellowship from the National Institute of Mental Health for a 15-month study at Karolinska Institute in Stockholm beginning May 16, 1973. Although the fellowship has been sought for the purpose of studying a new chemical method for application to brain neurotransmitter analysis ("mass fragmentography") during ethanol intoxication, it is apparent that the fellowship will offer a unique opportunity to study brain neurotransmitters during nicotine's action, as is the intent of the present CTR-USA project. The proposed extension of the present project is outlined in 8 below.

An extension of the present project would be unwarranted if it were not for the progress made during the first 7 months of the project. This progress is detailed in the accompanying progress report, but briefly, these are the accomplishments:

A. Discovery, through a large number of push-pull cannula experiments and leech muscle bioassays, that nicotine releases free acetylcholine from the rabbit hippocampus in a dose-response manner.
B. Discovery that large doses of mecamylamine, a central nicotinic antagonist, blocks the learning-facilitation action of nicotine, whereas small doses do not.
C. Discovery that nicotine, so far, does not produce a marked hippocampal theta rhythm in telemetry-monitored EEG, in doses which facilitate learning.

(continued on page 1a)

7. Give a Brief Statement of your Working Hypothesis:

Nicotine enhances learning by increasing the release of free acetylcholine in the hippocampus, that part of the brain which reputedly mediates learning.

(No change from previous hypothesis.)

Source: https://www.industrydocuments.ucsf.edu/docs/xxxl0000

6. Objectives or Specific Aims (Continued):

D. Rat-size push-pull cannulas have been obtained and are partially working, although we have no concrete data as yet on nicotine's effect on acetylcholine release in rats.

Thus, the present project as outlined in the earlier application is more than halfway completed. The results indicate that further studies on nicotine's effects on neurotransmitter release are warranted. It will be the objective of the extended project to utilize the equipment at Karolinska Institute to:

A. study the release of hippocampal norepinephrine in relation to hippocampal acetylcholine, in order to gain further insight into the specificity or non-specificity of nicotine in altering neurotransmitters, and
B. utilize the computerized behavioral system available at Karolinska to study apparent discrepancies in our atropine-nicotine data (see progress report for a discussion of the discrepancies).

(Details of the methods for carrying out the above objectives are given in 8 below.)

In support of the decision to look at hippocampal levels of norepinephrine, Hall and Turner (1972) have recently shown that nicotine injected intravenously in cats causes an increased release of norepinephrine from the hypothalamus. The question thus arises as to whether nicotine does the same thing in the hippocampus and whether such changes can be correlated with nicotine's enhancement of learning. Schechter and Rosecrans (1972) have proposed an interesting hypothesis that states that nicotine may act on a specific nicotine-sensitive cholinergic receptor in the CNS, which causes release of norepinephrine, along the lines of the Burn and Rand hypothesis. Such an hypothesis is extremely important in deciding the pharmacologic mechanism of nicotine's central effect. Looking at both acetylcholine and norepinephrine release from the hippocampus in the proposed experiments should provide interesting data to test Schechter and Rosecran's hypothesis.

10035845

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

The following experiments will be performed at Karolinska Institute (see Physical Facilities Available below for useable equipment):

A. Rabbit experiments - Large albino rabbits will be prepared in the same manner as in the original application (unanesthetized, immobilized with a neuromuscular blocker, and implanted with a push-pull cannula into the hippocampus). Perfusates, collected as described earlier, will be assayed for norepinephrine in addition to acetylcholine. The techniques for analyzing both of these chemicals by the "mass fragmentography" method are to be learned in Stockholm (see attached application for Special Fellowship). Acetylcholine and norepinephrine have been analyzed by this method in Holmstedt's laboratory (Hammar et al., 1968) and other laboratories (Koslow et al., 1972). Acetylcholine itself will first be studied by using the pyrolysis-gas chromatography method which is readily available in Holmstedt's laboratory, and later it will be studied using the more sophisticated mass fragmentography method, after it has been perfected by the applicant.

B. Rat experiments - The computerized behavioral equipment at Karolinska will provide an opportunity to study the problems we have been having with failure to

(see page 2a)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

(see page 2a)

10. Additional Requirements:

The items listed in the budget on page 3 can be justified as follows:

A. The full-time research technician is needed to work on the nicotine project because 100% of the applicant's time must be spent on satisfying the Special Fellowship objective, which is to learn the mass fragmentography technique and apply it to the identification of neurotransmitters during ethanol intoxication.
B. Supplies are required to perform the nicotine experiments. Although the Special Fellowship provides some supply money, this money can not be used to carry on the nicotine project.
C. The ½-time secretary, at the University of Kansas, would be extremely valuable in forwarding mail, typing one or two planned manuscripts on our present nicotine work, and keeping an account of the nicotine project supply funds. The department plans to obtain a temporary replacement for the applicant, and secretarial help in the department is not sufficient to take care of the applicant's needs as well as the replacement's needs.

1003538846

11. Biographical sketches of all principal and professional personnel (append)

There have been no significant changes in the biographical sketch of the principal investigator.

(see page 2b)

12. List of publications: (Five most recent as pertinent) (append)

(see page 2b)

Source: https://www.industrydocuments.ucsf.edu/docs/dryl0000

8. Details of Experimental Design and Procedures (Continued):

replicate our earlier observations that atropine blocks nicotine's enhancement of avoidance learning. Rats will be trained on the same discriminated lever-press avoidance schedule as we have been using, and the characteristics of their learning speed and performance will be analyzed by the on-line PDP-12 computer in an attempt to determine whether earlier atropine blockade of nicotine's effect was an artifact or a real pharmacologic action.

The computer has the advantage that it can show moment-to-moment performance of the animal so that we can observe whether nicotine has a short duration of action followed by a rebound (opposite) effect within an hourly training session. Generally, the behavior of the rat can also be "dissected" by the computer into individual components to provide further information on the effects of nicotine and atropine on the behavior.

References

Hall, G.H. and Turner, D.M., "Effects of nicotine on the release of ^{3}H-noradrenaline from the hypothalamus", Biochem. Pharmacol. 21: 1829-38 (1972).

Hammar, C.-G., Hanin, I., Holmstedt. B., Kitz, R.J., Jenden, D.J., and Karlen, B., "Identification of acetylcholine in fresh rat brain by combined gas chromatography-mass spectrometry", Nature 220: 915-17 (1968).

Hammar, C.-G., Holmstedt, B., Lindgren, J.-E., and Tham, R., "The combination of gas chromatography and mass spectrometry in the identification of drugs and metabolites", Adv. Pharmacol. Chemoth. 7: 53-89 (1969).

Koslow, S.H., Cattaberri, F., and Costa, E., "Norepinephrine and dopamine: assay by mass fragmentography in the picomole range", Science 176: 177-80 (1972).

Schechter, M.D. and Rosecrans, J.A., "Nicotine as a discriminative stimulus in rats depleted of norepinephrine or 5-hydroxytryptamine", Psychopharm. 24: 417-29 (1972).

Schmidt, D.E., Szilagyi, P.I.A., Alkon, D.L., and Green, J.P., "Acetylcholine: release from neural tissue and identification by pyrolysis-gas chromatography", Science 165: 1370-71 (1969).

9. Physical Facilities Available (Continued):

The following facilities, which will be available for my use, are located in the Department of Toxicology, Karolinska Institutet, Stockholm, Sweden and are under the direction of Professor Bo Holmstedt, Chairman of the department:

A. An LKB 9000 gas chromatograph-mass spectrometer ("mass fragmentographer") which is a sensitive instrument used for detecting small quantities of specific chemicals released from biological tissue (Hammar et al., 1969).
B. Two Series 1200 Varian gas chromatographs with pyrolyzers for analysis of small quantities of acetylcholine (Schmidt et al., 1969).
C. Two behavioral test chambers, with a PDP-12 computer for analysis of lever-press behavior inside the chambers.

1003538847

Source: https://www.industrydocuments.ucsf.edu/docs/ryby0000

D. Office space and desk.
E. A surgical area for push-pull cannula implantations.

11. Biographical sketches of all principal and professional personnel (Continued):

The research technician who will work on the project in Sweden has not yet been chosen. The only technique that we are now using that will be needed in Sweden is the rabbit push-pull perfusion method. This is capable of being learned by a competent research technician in one month. There is a chance that the technician now working on the project will be able to go to Sweden, which would obviously be advantageous to the project.

12. List of publications:

1. Erickson, C.K., Thorn, S. and Miller, T., "Studies on the mechanism of avoidance facilitation by nicotine", Abstracts of 116th Annual Meeting of the Amer. Pharm. Assoc., Montreal, Canada, pg. 105 (May, 1969).

2. Erickson, C.K. and Patel, J., "Facilitation of avoidance learning by post-trial hippocampal electrical stimulation", J. Comp. Physiol. Psych. 68: 400-6 (1969).

3. Erickson, C.K. and Burnam, W.L., "Cholinergic alteration of ethanol-induced sleep and death in mice", Agents and Actions 2: 8-13 (1971).

* 4. Erickson, C.K., "Studies on the mechanism of avoidance facilitation by nicotine", Psychopharmacologia 22: 357-68 (1971).

5. Erickson, C.K., "Facilitation of discriminated leverpress avoidance learning by ribaminol", Life Sci. 11: 23-32 (1972).

** 6. Erickson, C.K. and Graham, D.T., "Alteration of cortical and reticular acetylcholine release by ethanol in vivo", J. Pharmacol. Exp. Therap. (In Press, 1973).

* Copies recently sent to the Council.

** Copy submitted with this application.

10035388848

Downloaded from https://www.industrydocuments.ucsf.edu/docs/[illegible]

13. Budget: (1st year)

NOTE: (The grant will be administered by the University of Kansas, which will transfer funds for the items marked with * to Karolinska Institute as needed. The Business Officer whose signature appears below has approved this plan for the University.)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Technical		
*Research technician (12 months, including fringe benefits)	100%	$8,200
Secretarial help, to forward mail, type manuscripts, etc. ($2/hour)	10 hr/week	1,040
	Sub-Total	$9,240
B. Consumable Supplies (list by categories)		
*Gas chromatographic supplies		100
*Rabbits, rats, food, bedding		800
*Drugs and Chemicals		200
	Sub-Total	$1,100
C. Other Expenses (itemize)		
None		
	Sub-Total	
D. Permanent Equipment (itemize)		
None		
E. Overhead (15% of A+B+C)		$1,551
(15% of $10,340)	Total	$11,891

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Carlton K. Erickson

Director of Project Carlton K. Erickson

(913) 864-4002 Telephone

Signature Henry L. Snyder

Business Office of the Institution Henry L. Snyder

(913) 864-3126 Telephone

10035388 49

Source: https://www.industrydocuments.ucsf.edu/docs/fryl0000

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
1. "Effects of nicotine on free acetylcholine in the hippocampus during learning"	CTR-USA	$10,550	6/1/72 to 5/31/73
2. "Central neurotransmitter effects of ethanol"	National Institute on Alcohol Abuse and Alcoholism (NIMH)	$30,901	6/1/72 to 5/31/73
3. "Investigation into an animal model for familial dysautonomia"	University of Kansas General Research Fund	$2,720	7/1/72 to 6/30/73

Pending

1. Special Fellowship - "Analysis of neurotransmitters by mass fragmentography"	National Institute of Mental Health	Salary and supplies (to be determined)	5/16/73 to 8/15/74
2. "Search for a common mechanism of alcohol-antagonizing drugs"	University of Kansas General Research Fund	$4,100	7/1/73 to 6/30/74
3. "Relationship between alcohol-blocking drugs"	Licensed Beverage Industries, Inc.	$10,934	6/1/73 to 5/31/74

1003538850

Source: https://www.industrydocuments.ucsf.edu/docs/rryl0000